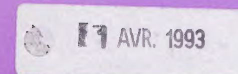


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ALYTES

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Descripción de una nueva especie europea de rana parda (Amphibia, Anura, Ranidae)

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This paper describes a new species of brown frog, discovered on the fringes of the Ordesa and Monte Perdido Park (Huesca, Spain). It is a slender, agile, long-legged and medium sized species with usually a light coloration, a small tympanum and well-separated nostrils, and it favours torrent habitats. It lives in clear, cold, oxygenated and reasonably fast-running waters where it can find refuge under stones or in tiny fissures. In most cases it is sympatric with *Euproctus asper* and, in some cases, with *Rana temporaria*. Its morphology and ecology distinguish it from *Rana temporaria*, *Rana iberica* and *Rana dalmatina*. Its area of distribution is limited, so far, to the Spanish side of the central and western Pyrenees.

A finales de verano de 1990, con motivo de un estudio faunístico sobre los vertebrados del Parque Nacional de Ordesa y Monte Perdido (provincia de Huesca, España) y su zona periférica, el autor capturó, por primera vez, la especie aquí descrita. Las ranas se hallaron en un torrente del valle de Ordiso. A partir de la referida fecha, se realizaron múltiples estudios y prospecciones periódicas para determinar y conocer el alcance del hallazgo. En invierno de 1991 se logró encontrar diversas puestas de dicha especie, siendo fotografiadas y trasladadas al laboratorio para su ulterior estudio ontogénico. Posteriormente, la realización de campañas prospectivas permitió hallar diecisiete localidades distintas para la nueva rana, algunas de ellas muy alejadas entre sí. Los resultados obtenidos después de dos años de estudios, indican que las ranas halladas pertenecen a una nueva especie.

El presente artículo describe la morfología y algunas de las características ecológicas de la nueva rana, destacando sus principales rasgos distintivos. Su morfometría, ecología, biología, diferencias aloenzimáticas, cariotipo y desarrollo larvario se tratarán más exhaustivamente en oportunas ulteriores publicaciones, algunas de ellas ya en curso

(artículo en preparación sobre morfometría elaborado por DUBOIS, OHLER & SERRA-COBO).

Abreviaciones utilizadas. — MNCN: Museo Nacional de Ciencias Naturales de Madrid (CSIC); MNHN: Muséum National d'Histoire Naturelle, Paris; MZB: Museu de Zoologia de Barcelona; DZV: Departament de Biologia Animal, Fac. Biologia, Universitat Barcelona; IPE: Instituto Pirenaico de Ecología (CSIC), Jaca-Zaragoza. La notación utilizada para cada variable, viene indicada en el pie de la Tabla I.

Material de comparación. — Dieciocho adultos de *Rana iberica* (MNHN 1992.4852-4853, 4857, 4859-4860, 4863, 4865, 4870-4873, 4875, 4878, 4881-4883, 4885-4886) de la Laguna Grande de Gredos (Ávila, España). Nueve machos adultos de *Rana temporaria* (MNHN 1979.7510-7514, 7516-7517, 7520, 7522) del Col du Pourtalet (Pyrénées Atlantiques, Francia). Diez machos de *Rana dalmatina* (MNHN 1980.1009-1016, 1019-1020) de la Forêt de Fontainebleau (Seine-et-Marne, Francia).

Metodología estadística utilizada. — Todas las variables, excepto SVL, han sido transformadas en tanto por mil de la longitud hocico-ano antes de ser procesadas estadísticamente. La Tabla I se ha elaborado a partir de las medidas del holotipo y de los paratipos (8 hembras y 15 machos). Para comparar las distintas especies se ha considerado como unidad de tratamiento los individuos adultos de una misma población y mismo sexo (machos capturados en la Espata: MNCN 16661; MNHN 1993.2502, 2504, 2509-2511, 2514-2516), utilizando el test estadístico de la U de Mann-Whitney (DUBOIS, 1984a; SIEGEL, 1985; OHLER & KAZADI, 1990).

Rana pyrenaica sp. nov.

(fig. 1-4)

Holotipo. — MNCN 16661, macho adulto capturado el 12 de octubre de 1992, por Jordi SERRA-COBO, en la Espata (Villanua, Huesca, España).

Medidas del holotipo. — SVL = 45,3 mm; HW = 339; HL = 337; IFE = 174; FLL = 245; TL = 546; FOL = 574; IN = 86; EN = 71; EL = 91; TYD = 35; TFL = 124; IMT = 42; ITL = 124; 2TL = 209; 4TL = 515; TT = 253; ABL = 195; LCD = 497; EE = 62.

Paratipos. — Siete hembras adultas (MNHN 1992.5234-5235, 1993.2501, 2507, 2512-2513; DZV 2766) y siete machos adultos (MNHN 1993.2502, 2504, 2509-2511, 2514-2515) capturados el 14 de julio de 1992, y un macho adulto (MNHN 1993.2516) capturado el 12 de octubre de 1992, por Jordi SERRA-COBO, en la Espata (Villanua, Huesca, España). Dos machos adultos (MNHN 1993.2506, 1992.5236) capturados el 10 y el 12 de octubre de 1992, respectivamente, por Jordi SERRA-COBO, en el valle de Bujaruelo (Torla, Huesca, España). Un macho adulto (MNHN 1993.2505) capturado el 11 de setiembre de 1990, dos machos adultos (MNHN 1993.2503, 2508) capturados el 15 de julio de 1992, y una hembra adulta (MZB 92.0167) y un macho adulto (IPE 1992.4040) capturados el 12 de octubre de 1992, por Jordi SERRA-COBO, en el valle de Ordiso (Torla, Huesca, España).

Etimología del nombre específico. — Hace referencia a la cordillera donde se descubrió la especie.

Tabla I. — Resumen de los datos biométricos obtenidos para 8 hembras y 15 machos de *Rana pyrenaica*.

$\bar{X}$, media; σ, desviación estandard; V_H, coeficiente de variación de Haldane (Haldane, 1955; Delaugerre & Dubois, 1985); U, resultado del test de Mann-Whitney (comparación hembras-machos); P, probabilidad asociada al valor de U para un test bilateral. Variables estudiadas: SVL, longitud morro-cloaca; HW, anchura de la cabeza; HL, longitud de la cabeza; IFE, separación entre la parte anterior de los ojos; FLL, longitud del antebrazo (desde el codo al borde proximal del tubérculo palmar externo); TL, longitud de la pierna (desde la articulación tibio-tarsal a la tibio-femoral); FOL, longitud del pie (desde el borde proximal del tubérculo metatarsiano interno hasta el extremo del cuarto dedo); IN, separación entre orificios nasales; EN, distancia entre borde anterior del ojo y orificio nasal; EL, longitud del ojo; TYD, diámetro del tímpano; TFL, longitud tercer dedo de la mano (de la base primer tubérculo al extremo del dedo); IMT, longitud del tubérculo metatarsiano interno; ITL, longitud primer dedo del pie; IMT/ITL, tanto por mil del tubérculo metatarsiano interno respecto a la longitud del primer dedo del pie; 2TL y 4TL, longitudes del 2° y 4° dedos del pie, respectivamente (medidos desde el borde distal del tubérculo metatarsiano interno, hasta el extremo del dedo); TT, longitud del talón (desde articulación tibio-tarsal al borde proximal del tubérculo metatarsiano interno); ABL, longitud del brazo (medido por la cara ventral); LCD, longitud del muslo (de la cloaca a la rodilla); EE, distancia interorbitaria.



	Hembras					**Machos**						
Variable	**Rango**	**$\bar{X}$**	**σ**	**Mediana**	**V_H**	**Rango**	**$\bar{X}$**	**σ**	**Mediana**	**V_H**	**U**	**P**
SVL	35,5-51,0	43,3	4,49	42,9	10,69	33,4-45,7	42,0	3,31	43,1	8,01	51	>0,10
HW	323-410	359	26,86	361,5	7,72	305-365	350	16,97	353	4,93	48,5	>0,10
HL	329-368	346	14,38	346	4,29	309-357	334	13,15	337	4,00	36	>0,10
IFE	162-198	173	12,12	168,5	7,22	166-191	175	8,20	174	4,76	45	>0,10
FLL	212-274	243	21,54	249	9,14	222-281	258	16,96	262	6,68	37	>0,10
TL	518-588	557	20,07	557	3,72	543-619	565	22,87	557	4,12	57,5	>0,10
FOL	497-582	550	31,36	562,5	5,88	539-642	579	23,28	579	4,09	25	<0,05
IN	88-101	93	4,99	90,5	5,53	84-100	91	5,10	90	5,70	46	>0,10
EN	71-79	75	3,25	74,5	4,47	62-84	73	5,18	72	7,21	43	>0,10
EL	96-123	109	9,70	108,5	9,18	88-124	106	12,34	105	11,83	50,5	>0,10
TYD	31-48	41	5,46	41	13,73	26-47	36	5,78	36	16,32	33	=0,10
TFL	126-144	136	6,29	135,5	4,77	110-139	128	8,33	128	6,62	27	<0,05
IMT	33-50	42	5,71	40,5	14,02	33-51	42	4,77	42	11,55	56	>0,10
ITL	90-124	111	11,01	113,5	10,23	93-128	117	10,22	121	8,88	37,5	>0,10
IMT/ITL	279-480	370	65,24	385,5	18,18	260-494	366	63,24	351	17,57	60	>0,10
2TL	173-228	212	16,72	214	8,13	182-239	222	16,04	227	7,35	35,5	>0,10
4TL	465-563	511	31,06	517	6,27	480-569	528	28,44	528	5,48	41	>0,10
TT	220-273	251	20.51	252,5	8,43	239-298	257	14,60	251	5,78	52	>0,10
ABL	162-225	201	20,23	206,5	10,38	171-244	206	22,82	209	11,26	50	>0,10
LCD	469-565	517	32,18	515,5	6,42	461-561	510	25,71	509	5,13	53	>0,10
EE	57-93	74	11,52	73	16,05	62-90	73	8,98	72	12,51	55	>0,10

Tabla II. — Comparación morfométrica entre 9 machos adultos de *Rana pyrenaica* y 18 machos adultos de *Rana iberica*. Se considera P la probabilidad asociada al valor de U para un test bilateral.

Especie	Rango	$\bar{X}$	σ	V_H	Mediana	U	P
	Longitud del antebrazo (en ‰ de SVL)						
R. pyrenaica	245-277	260	13,08	5,17	262		
R. iberica	202-304	236	21,10	9,36	234	13	<0,002
	Diametro máximo del tímpano (en ‰ de SVL)						
R. pyrenaica	26-47	38	5,89	15,93	39		
R. iberica	42-70	52	7,73	15,08	51,5	8	<0,002
	Longitud del tercer dedo de la mano (en ‰ de SVL)						
R. pyrenaica	110-139	125	8,24	6,78	127		
R. iberica	143-178	164	8,74	5,48	164,5	0	<0,002
	Longitud del primer dedo del pie (en ‰ de SVL)						
R. pyrenaica	93-128	116	11,18	9,91	121		
R. iberica	112-151	133	8,58	6,54	133,5	13	<0,002
	Longitud del segundo dedo del pie (en ‰ de SVL)						
R. pyrenaica	182-239	216	18,06	8,59	217		
R. iberica	222-259	244	9,87	4,10	247,5	11	<0,002
	Longitud del talón (en ‰ de SVL)						
R. pyrenaica	243-298	259	17,05	6,77	251		
R. iberica	228-281	245	13,21	5,47	242,5	38	<0,02
	Distancia interorbitaria (en ‰ de SVL)						
R. pyrenaica	62-86	71	8,42	12,19	72		
R. iberica	85-112	95	6,41	6,84	95	1	<0,002

Diagnosis. — Especie de cuerpo grácil y talla mediana, inferior a *Rana temporaria* y *R. dalmatina* (Tablas III y IV). Orificios nasales más separados entre sí y proporcionalmente más distantes del borde anterior del ojo que en *R. temporaria* y *R. dalmatina* (Tablas III y IV). Mancha temporal poco conspicua, menos que en *R. iberica*, *R. temporaria* y *R. dalmatina*. Tímpano muy pequeño y difícil de distinguir (Tabla I). Hocico menos acuminado que en *R. iberica*. Antebrazo y pierna relativamente largos respecto a la talla del cuerpo (Tabla I). Tercer dedo de la mano relativamente menor que en *R. iberica* y *R.*

Tabla III. – Comparación morfométrica entre 9 machos adultos de *Rana pyrenaica* y 9 machos adultos de *Rana temporaria*. Se considera P la probabilidad asociada al valor de U para un test bilateral.

Especie	Rango	$\bar{X}$	σ	V_H	Mediana	U	P
	Longitud del hocico-ano (en ‰ de SVL)						
R. pyrenaica	39,0-45,7	43,2	2,50	5,95	43,8		
R. temporaria	58,9-70,3	65,8	3,20	5,00	65,9	0	<0,002
	Separación entre la parte anterior de los ojos (en ‰ de SVL)						
R. pyrenaica	167-176	172	3,76	2,25	174		
R. temporaria	133-150	145	5,03	3,57	146	0	<0,002
	Longitud de la pierna (en ‰ de SVL)						
R. pyrenaica	543-564	552	6,97	1,30	552		
R. temporaria	513-555	533	15,84	3,05	530	11	<0,02
	Separación entre orificios nasales (en ‰ de SVL)						
R. pyrenaica	86-100	90	4,58	5,23	89		
R. temporaria	68-80	72	4,22	6,02	72	0	<0,002
	Distancia entre orificio nasal y borde anterior del ojo (en ‰ de SVL)						
R. pyrenaica	62-79	71	4,93	7,14	70		
R. temporaria	54-68	63	4,19	6,84	64	6	<0,002
	Diámetro máximo del tímpano (en ‰ de SVL)						
R. pyrenaica	26-47	38	5,89	15,93	39		
R. temporaria	56-77	70	6,12	8,99	70	0	<0,002
	Longitud del segundo dedo del pie (en ‰ de SVL)						
R. pyrenaica	182-239	216	18,06	8,59	217		
R. temporaria	230-280	257	18,92	7,57	257	4	<0,002

dalmatina (Tablas II y IV). Pierna relativamente más larga que en *R. temporaria* (Tabla III) y menos que en *R. dalmatina* (Tabla IV). Articulación tibio-tarsal rebasando el extremo del hocico cuando se dobla la extremidad posterior hacia adelante. Longitud del primero y segundo dedos del pie menor que en *R. iberica* (Tabla II). Dedos de las manos y de los pies redondeados en su extremo distal. Coloración de la garganta normalmente jaspeada gris ténue y sin línea media clara como en *R. iberica*. Coloración dorsal entre canela crema y gris oliváceo. Canto grave y débil, más flojo que en *R. temporaria*.

Tabla IV. — Comparación morfométrica entre 9 machos adultos de *Rana pyrenaica* y 10 machos adultos de *Rana dalmatina*. Se considera P la probabilidad asociada al valor de U para un test bilateral.

Especie	Rango	$\bar{X}$	σ	Vн	Mediana	U	P
	Longitud del hocico-ano (en ‰ de SVL)						
R. pyrenaica	39,0-45,7	43,2	2,50	5,95	43,8		
R. dalmatina	48,9-61,4	56,1	3,84	7,02	56,7	0	<0,002
	Separación entre la parte anterior de los ojos (en ‰ de SVL)						
R. pyrenaica	167-176	172	3,76	2,25	174		
R. dalmatina	147-163	154	5,64	3,75	154	0	<0,002
	Longitud de la pierna (en ‰ de SVL)						
R. pyrenaica	543-564	552	6,97	1,30	552		
R. dalmatina	607-631	618	8,24	1,37	618,5	0	<0,002
	Longitud del pie (en ‰ de SVL)						
R. pyrenaica	539-591	567	15,97	2,13	574		
R. dalmatina	571-603	588	12,21	2,13	588,5	13	<0,02
	Separación entre orificios nasales (en ‰ de SVL)						
R. pyrenaica	86-100	90	4,58	5,23	89		
R. dalmatina	71-78	74	2,23	3,09	74	0	<0,002
	Distancia entre orificio nasal y borde anterior del ojo (en ‰ de SVL)						
R. pyrenaica	62-79	71	4,93	7,14	70		
R. dalmatina	60-72	65	3,99	6,29	65	13,5	<0,02
	Diámetro máximo del timpano (en ‰ de SVL)						
R. pyrenaica	26-47	38	5,89	15,93	39		
R. dalmatina	71-88	78	6,43	8,45	75,5	0	<0,002
	Longitud del tercer dedo de la mano (en ‰ de SVL)						
R. pyrenaica	110-139	125	8,24	6,78	127		
R. dalmatina	130-143	136	5,15	3,88	136,5	6,5	<0,002
	Longitud del cuarto dedo del pie (en ‰ de SVL)						
R. pyrenaica	480-542	510	19,06	3,84	505		
R. dalmatina	513-560	537	15,94	3,04	541	13	<0,02
	Longitud del muslo (en ‰ de SVL)						
R. pyrenaica	476-524	503	14,66	3,00	502		
R. dalmatina	539-585	570	16,44	2,96	576,5	0	<0,002
	Longitud del talón (en ‰ de SVL)						
R. pyrenaica	243-298	259	17,05	6,77	251		
R. dalmatina	268-292	279	7,01	2,58	278	12	<0,02

Descripción morfológica del adulto. — Es un anuro de talla mediana (Tabla I) y de cuerpo esbelto. La cabeza es ligeramente más ancha que larga (Tabla I). La distancia entre los orificios nasales es superior a la separación interorbitaria (Tabla I). Las oberturas nasales están más cercanas al extremo del hocico que al borde anterior del ojo (fig. 1). El hocico es corto, no acuminado, proyectándose ligeramente por delante de la obertura bucal. La mancha temporal es poco conspicua, prolongándose, en franja estrecha, por delante del ojo hasta alcanzar el extremo del morro. La parte posterior de la referida mancha está delimitada por dos pliegues prominentes: uno supratemporal y otro subtemporal. Los ojos, no muy saltones, se disponen lateralmente. La pupila es oval. El canthus rostralis es romo. Sobre el labio superior un repliegue de coloración clara se prolonga hacia la región subtemporal, rebasando la comisura bucal y, en algunos casos, alcanzando la base de las extremidades anteriores. En la mayoría de individuos dicha franja clara se inicia cerca del extremo del hocico. El tímpano es pequeño y de difícil observación (Tabla I), rasgo característico de la especie (fig. 1-2). Presenta dos series de dientes vomerianos situadas detrás de las coanas y en posición oblicua respecto al eje del cuerpo, más separadas entre sí en su extremo distal que en su extremo proximal. La separación entre el extremo distal de los dientes vomerianos y las coanas es menor que la longitud de las series dentarias.

Los miembros anteriores son relativamente largos (sobre todo el antebrazo) y permiten el levantamiento del tórax y la cabeza. El antebrazo es más largo que el brazo (Tabla I). El tercer dedo de la mano es corto (Tabla I), aproximadamente la mitad del antebrazo. El extremo distal de los dedos de la mano es redondeado. Los tubérculos subarticulares son romos o ligeramente acuminados, circulares y están bien desarrollados. Las manos presentan dos tubérculos palmares alargados (fig. 3).

Las extremidades posteriores son relativamente largas, sobre todo la pierna, respecto a la talla del cuerpo. Al doblarlas hacia adelante, la articulación tibio-tarsal apenas rebasa el extremo del hocico. El pie (desde el borde proximal del tubérculo metatarsiano interno hasta el extremo del cuarto dedo) es aproximadamente igual a la longitud de la pierna (Tabla I). Los dedos del pie presentan su extremo distal redondeado. La membrana interdigital es fina y está muy desarrollada, incluyendo en ella la mayor parte de todos los dedos, excepto la porción distal del cuarto dedo. El tubérculo metatarsiano interno es pequeño, romo, oval y de consistencia blanda, siendo su longitud alrededor de 1/4 a 1/2 de la talla del primer dedo. Los tubérculos subarticulares están, en general, bien desarrollados y son circulares, romos o ligeramente acuminados (fig. 4).

La piel es lisa, fina y muy viscosa en animales vivos. Los repliegues dorso-laterales son estrechos, prolongándose desde la parte posterior del ojo hasta la región anal.

Coloración. — La coloración dorsal varía entre color canela crema y gris oliváceo, presentando discretas manchas verdosas. Sin embargo, las referidas moteaduras son poco conspicuas en animales vivos, ora en la región dorsal, ora en las extremidades anteriores y posteriores. Algunos ejemplares pueden presentar una mancha en forma de V invertida en la espalda. Las hembras suelen tener ciertos tonos rojizos en los párpados, alrededor de los repliegues dorso-laterales y en la región dorsal de las extremidades posteriores, y su cuerpo suele ser más voluminoso. No obstante, en algunas ocasiones se han observado machos con tonalidades rojizas, si bien éstas eran más tenues (holotipo MNCN 16661). El

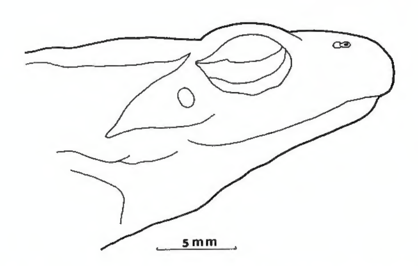


Fig. 1.- *Rana pyrenaica* sp. nov., visión lateral de la cabeza (MNHN 1992.5236).

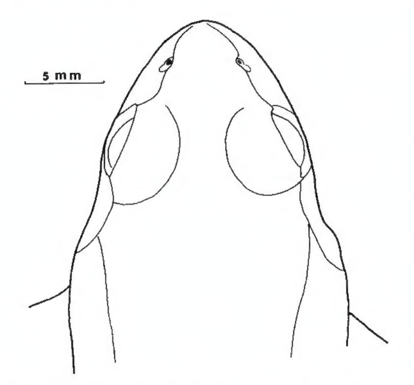


Fig. 2. – *Rana pyrenaica* sp. nov., visión dorsal de la cabeza (MNHN 1992.5236).

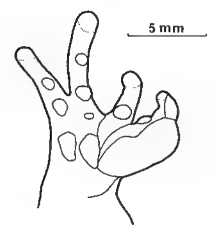


Fig. 3. — *Rana pyrenaica* sp. nov., visión ventral de la mano derecha (MNHN 1992.5236).

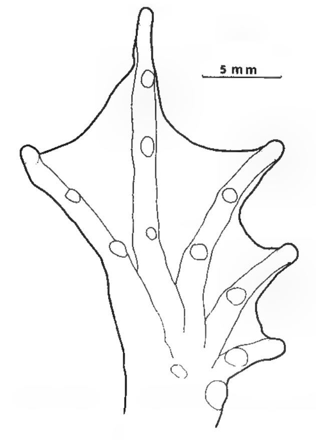


Fig 4. — *Rana pyrenaica* sp nov., visión ventral del pie derecho (MNHN 1992 5236).

iris es dorado con pequeñas puntuaciones de color oscuro. La región ventral es muy clara, existiendo, casi siempre, un ligero jaspeado gris-rosáceo en la garganta, pero en ningún caso se ha observado una banda clara en la línea media, ni tampoco presenta una banda transversal. La coloración ventral a nivel femoral es amarillenta, pudiendo ser azulada en verano. Durante la época de celo la región femoral de los machos es sonrosada. La pigmentación tiende a ser bastante homogénea en todos los ejemplares estudiados.

Caracteres sexuales secundarios. — La longitud del tercer dedo de la mano suele ser mayor en las hembras que en los machos (Tabla I). La longitud del pie tiende a ser mayor en los machos que en las hembras (Tabla I). Los machos carecen de sacos bucales y su canto es grave y tenue. Las callosidades palmares de los machos son, generalmente, amarillentas (fig. 3). Después del letargo, los machos presentan desarrollados pliegues cutáneos en la región lateral del tronco, en la región dorso-posterior de la cabeza y a nivel femoral, los cuales devienen conspicuos durante el amplexus.

Puesta y desarollo. — La puesta se efectúa entre finales de invierno y principios de primavera (febrero-marzo), dependiendo de las características climatológicas del biotopo, la altitud de la localidad de puesta y las oscilaciones meteorológicas estacionales. Las puestas son pequeñas, pues suelen estar constituidas por un número de huevos comprendido entre 70 y 110 (se han observado unas 15 puestas), y no forman masas compactas sino más bien racimos. La gelatina envolvente es muy densa, impidiendo la flotación. Por el contrario es escaso su espesor protector. La especie tiende a adherir la puesta bajo las piedras (un 87 % de las puestas observadas), en la vegetación de fondo o en las fisuras rocosas de arroyos y, ocasionalmente, sobre fondo lodoso siempre que las aguas estén quietas. El embrión es de color pardo-oscuro y relativamente grande respecto a su envoltura gelatinosa.

La velocidad de desarrollo embrionario está en función de las condiciones climatológicas anuales, siendo frecuente el retraso ontogénico como consecuencia de las nevadas y fríos primaverales tardíos, típicos de la región pirenaica occidental. Transcurridas unas semanas, el embrión pierde su intensa coloración oscura, adquiriendo un tono grisáceo claro al llegar al estadio en que se forman los esbozos de la cabeza y la cola. Llama la atención el relativo gran desarrollo de su abdomen y su delgada y delicada piel La cola adquiere rápidamente una notable longitud relativa.

Las larvas ya desarrolladas son muy características y fáciles de distinguir por su coloración oscura, su musculosa aleta caudal y sus manchas de color dorado con irizaciones (pueden ser más o menos moteadas según las poblaciones). Tienen aspecto robusto y a su vez estilizado y elegante. Están bien adaptadas a los cursos de agua, disponiéndose en su fondo al amparo de la corriente o nadando a modo de "trucha"

Distribución y ecología. — Los adultos y subadultos se alejan poco del agua, situándose en riberas de arroyos o bien escondiéndose bajo pequeños saltos de agua, piedras situadas en torrentes o en estrechas fisuras La especie tiende a ser simpátrica con *Euproctus asper*, siendo muy similares sus residencias ecológicas (un 82 % de las 17 localidades donde se ha hallado a *R. pyrenaica* también habitaba *E. asper*). Suele vivir en aguas claras, frias y oxigenadas, ora pequeños cursos hídricos con poca renovación (siendo simpátrica con *R. temporaria* y desovando ambas especies en el mismo lugar), ora en torrentes caudalosos.

R. pyrenaica no se ha hallado nunca en aguas estancas Su gracilidad, agilidad, estrategia reproductora y anatomía de sus larvas, le permiten poblar biotopos donde a *R. temporaria* y al resto de anuros montanos pirenaicos les es difícil residir. Escapa, así, a una posible competencia trófica con la rana bermeja, *Bufo bufo* y *Alytes obstetricans*, anuros abundantes en la región centro-occidental del Pirineo. De las diecisiete localidades estudiadas, sólo en tres de ellas la especie es simpátrica con *R. temporaria*. Los efectivos de *R. pyrenaica* suelen dispersarse a lo largo de los cursos de agua, ora referente a los individuos adultos, ora a las larvas cuyas concentraciones no suelen ser tan elevadas como en *R. temporaria*. Dicha menor densidad de renacuajos, cabe atribuirla, en parte, a la estrategia reproductora seguida por *R. pyrenaica*, la cual tiende a ser de la K (como en *Alytes obstetricans*) y no de la r como en *R. temporaria* y *Bufo bufo*, y a la referida dispersión de los individuos adultos reproductores (BEGON, HARPER & TOWNSEND, 1988). Se ha hallado, a *R. pyrenaica*, en la franja altitudinal comprendida entre los 1200 y 1700 m s.m. Su distribución se circunscribe, por ahora, a la región pirenaica centro-occidental española (fig. 5).

Discusión — La ecología, morfología y etología de *R pyrenaica* le distinguen bien de *R. temporaria*, especie muy abundante en el Pirineo (BOULENGER, 1879, 1898, 1910; LANTZ, 1927; BECK, 1943; BALCELLS, 1956; MERTENS & WERMUTH, 1960; FRETEY, 1975; ARNOLD & BURTON, 1978, ANDRADA, 1980; PARENT, 1981; DUBOIS, 1982a-b, MARTÍNEZ-RICA,1983; MARTÍNEZ-RICA & REINÉ-VIÑALES, 1988; ESTEBAN, 1990). Menor talla del cuerpo, tímpano más pequeño, mancha temporal mucho más tenue, parte distal del hocico proporcionalmente más ancha, mayor separación, en relación a la longitud del cuerpo, entre orificios nasales y entre estos últimos y el borde distal del ojo, talla relativa de la pierna suele ser más larga (excepto en relación a la rana de Gasser, según estudio morfométrico realizado por DUBOIS, OHLER & SERRA-COBO, actualmente en preparación), menor longitud del segundo dedo del pie respecto a la talla del cuerpo y distinta coloración, son las principales características externas que permiten distinguir *R. pyrenaica* de *R. temporaria* (Tabla III). A ellas cabe añadir las diferencias existentes entre las puestas y las larvas de ambas especies. Las puestas de la nueva rana presentan menor número de huevos, no flotan (en *R. temporaria* sí) y el diámetro de los embriones es relativamente mayor respecto a la densa envoltura gelatinosa. Por otra parte, los renacuajos de *R. pyrenaica* siempre son mucho más obscuros, musculosos y con aleta caudal distinta (la parte distal suele ser más lobulada). Igualmente, es diferente la etología reproductora· *R. temporaria* suele desovar en aguas quietas, normalmente estancas, mientras *R pyrenaica* es mucho más selectiva y suele poner bajo piedras o en pequeñas grietas de arroyos y torrentes La mayoría de los rasgos distintivos, mencionados unas líneas más arriba, entre el nuevo taxón y *R. temporaria*, sirven también para diferenciar *R. pyrenaica* (especie mucho más pequeña) de *R. temporaria parvipalmata* Seoane, 1885, y de la rana de Gasser (DUBOIS, 1982b, 1983). No obstante, las referidas diferencias serán tratadas ampliamente en otro artículo (DUBOIS, OHLER & SERRA-COBO, en preparación).

Las poblaciones simpátricas de *R. pyrenaica* y *R. temporaria* tienen gran interés ecológico y biológico, pues confirman la diferenciación interespecífica. Las localidades donde cohabitan ambas especies, presentan siempre aguas quietas con cierto flujo renovador que impide el estancamiento hídrico. Si *R. pyrenaica* y *R. temporaria* fuesen morfos distintos de una misma especie adaptados a diferentes condiciones ecológicas, la

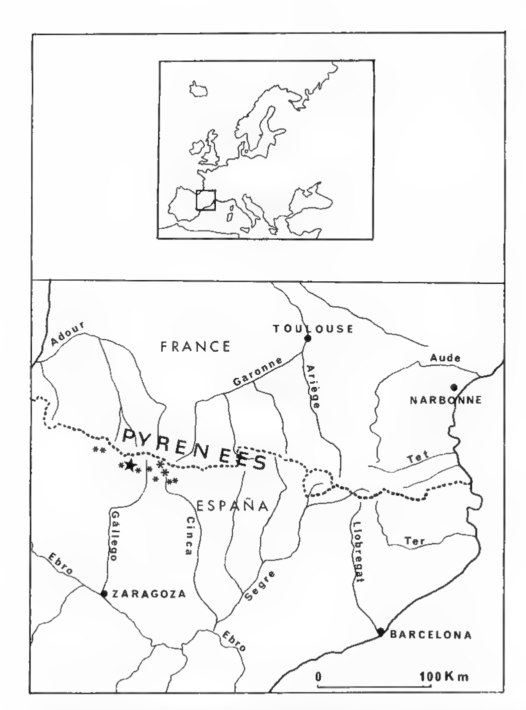


Fig. 5. – Distribución de *Rana pyrenaica* conocida hasta la fecha La estrella corresponde a la localidad tipo, mientras los asteriscos indican las localidades donde se ha hallado la especie. El asterisco mayor agrupa diversas localidades próximas entre sí.

variabilidad de los individuos pertenecientes a poblaciones simpátricas sería escasa, como resultado de fenómenos de hibridación (habría flujo génico entre poblaciones). No obstante, en biotopos donde existe simpatría, los individuos adultos, las puestas y las larvas de ambos taxones continuan diferenciándose claramente, manteniendo las características propias de las poblaciones alopátricas. Cabe descartar, así, que se produzca hibridación entre las dos especies y que la nueva rana sea fruto del acusado polimorfismo de *R. temporaria*. Por otra parte, se ha constatado que en las localidades simpátricas, las larvas de *R. temporaria* tienden a eclosionar más tarde. Así mismo, las observaciones realizadas, tanto "in situ" como en laboratorio, parecen indicar que el desarrollo ontogénico es más lento en *R. pyrenaica*.

La morfología de *R. pyrenaica* también es distinta respecto a *R. iberica*, especie, esta última, que si bien ha sido citada en repetidas ocasiones en los Pirineos (BELLOC, 1893; PLANTADA Y FONOLLEDA, 1903; SAGARRA, 1916; MALUQUER, 1916; BOSCÀ & CASANOVES, 1918; LANTZ, 1927; BECK, 1942, 1943; ANGEL, 1946, 1947; DOTTRENS, 1963; SALVADOR, 1974; FRETEY, 1975; CASTANET, 1978; CASTANET & GUYÉTANT, 1991; BERTRAND & CROCHET, 1992), los especialistas actuales ponen en duda su existencia en dicha cordillera (DUBOIS, 1982b; MARTÍNEZ-RICA, 1983). Teniendo en cuenta la referida opinión, actualmente no habrían áreas pirenaicas simpátricas para ambas especies. En comparación con *R. iberica*, *R. pyrenaica* presenta el hocico menos acuminado, la mancha temporal no tan conspicua, el tímpano más pequeño (Tabla II), la coloración de la garganta menos oscura y sin línea media clara. En relación a la longitud del cuerpo el tercer dedo de la mano es más corto, el antebrazo es más largo, el primer y segundo dedos del pie son proporcionalmente de menor talla, el talón tiende a ser mayor y la distancia interorbitaria es menor (Tabla II). Lamentablemente, los estudios sobre reproducción y desarrollo embrionario de *R. iberica* son muy escasos (CRESPO & CEI, 1973) e impiden un análisis comparativo.

Respecto a *R. dalmatina*, el anuro aquí descrito presenta talla más pequeña, mayor separación entre el borde anterior de ambos ojos, orificios nasales más separados entre sí, mayor distancia entre orificio nasal y borde distal del ojo, diámetro timpánico mucho menor, mancha temporal menos conspicua, parte inferior del iris más clara (ver DUBOIS, 1984b), hocico menos acuminado, tercer dedo de la mano más corto, menor longitud del muslo, de la tibia, del pie y del talón, el cuarto dedo del pie más corto y, en general, aspecto del cuerpo menos robusto (Tabla IV). Las puestas contienen menor número de huevos. Por otra parte, *R. pyrenaica* tiene hábitos más acuáticos. Hasta la fecha no se ha comprobado la presencia de *R. dalmatina* en los Pirineos, si bien se ha citado repetidamente en la literatura (DESPAX, 1941; BECK, 1942, 1943; ANGEL, 1946, 1947; HVASS, 1972).

El hallazgo, en Europa, de una especie vertebrada completamente nueva para la ciencia con caracteres morfológicos que permiten distinguirla en el campo (sin necesidad de recurrir a técnicas más complejas de laboratorio), no deja de ser un hecho sorprendente en nuestros días.

RESUMEN

El artículo describe un nuevo anuro, *Rana pyrenaica*, hallado en la zona periférica del Parque Nacional de Ordesa y Monte Perdido (Huesca, España). Es una rana parda grácil,

ágil, de mediana talla, coloración normalmente clara, tímpano pequeño, orificios nasales separados, patas relativamente largas, segundo dedo del pie corto, hábitos torrentícolas y que suele frecuentar aguas claras, frías, oxigenadas y más o menos rápidas, refugiándose bajo piedras o en pequeñas fisuras. En la mayoría de los casos es simpátrica con *Euproctus asper* y en ciertas ocasiones con *Rana temporaria*. Su morfología y ecología le distinguen de *R. temporaria*, *R. iberica* y *R. dalmatina*. Su repartición se circunscribe, hasta la fecha, a la vertiente española del Pirineo centro-occidental.

Agradecimientos

El autor agradece la colaboración prestada por el Dr. E. Balcells, tanto a nivel de prospecciones de campo, como en seguimientos de desarrollo larvario en el laboratorio. Cabe agradecer también al Prof. A. Dubois sus inestimables consejos e informaciones facilitadas, así como reconocer el tiempo dedicado por A. Ohler, J. Magraner y R. Bour a proporcionar material de comparación, información bibliográfica e informática. Expresar igualmente gratitud, por el soporte logístico de toda índole, al Laboratoire d'Ecologie de l'Ecole Normale Supérieure (CNRS URA-258) y en particular al Prof. R. Barbault. A B. Amengual, M. Lopez, C. Pedrocchi, L. Mas y J. E. Gomez agradecer su colaboración en la labor de campo. Finalmente, citar al P. N. de Ordesa y a la Diputación General de Aragón los cuales facilitaron el apoyo logístico que permitió el hallazgo de *R. pyrenaica* y al Ministerio de Educación y Ciencia (becas posdoctorales de formación en el extranjero) y a la Comunidad Europea (Human Capital and Mobility Programme, Community training project) quienes financiaron parte de su ulterior estudio. La sede operativa de la investigación tuvo lugar en el Centre d'Ecologie Montagnarde de Gabas (Université de Bordeaux I).

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Growth and maturity of brown frogs, *Rana arvalis* and *Rana temporaria*, in central Poland

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Growth of two brown frog species, *Rana arvalis* and *Rana temporaria*, was studied in nature and in captivity. In both species, growth was fastest in the first two years, maturity was reached in the second year, and gametes were released after the second hibernation. Individuals of *Rana arvalis* matured earlier than those of *Rana temporaria*, and in some males of *Rana arvalis* the sexual features were visible already before the first hibernation.

Introduction

The two species of brown frogs *Rana temporaria* and *Rana arvalis* belong to the common amphibians in central Europe. Data on their growth and sexual maturity are numerous, but opinions on this subject are controversial. Krivosheyev, Openko & Shabanova (1960) suggested that they grow most rapidly in the third (*Rana arvalis*) or in the fourth year (*Rana temporaria*), that is, in the year of reaching maturity. Most other authors state that they grow very quickly in the first and in the second year, whereas growth decreases after maturation (Gislén & Kauri, 1959; Kleinenberg & Smirina, 1969; Tomasik, 1969; Van Gelder & Oomen, 1970; Loman, 1978; Gibbons & McCarthy, 1984; Chmelevskaja, 1985; Ryser, 1988; among others). They report that the frogs can reach maturity between the second and the fourth year of their lives and release gametes for the first time after two to four hibernations.

In 1965 we found a surprisingly small adult *Rana arvalis* male (37.5 mm, snout-vent length) in a breeding aggregation, and in 1977 we caught a very small female of *Rana arvalis* (39.5 mm) in amplexus, which subsequently laid 404 eggs in an aquarium. These data prompted us to observe brown frogs more precisely.

In the present paper we review available data and compare them with our results, which suggest that growth and sexual maturity in these frog species depend not only on geographical distribution, but also on environmental conditions.

MATERIAL AND METHODS

This paper on brown frogs arose as a by-product during the study of European water frogs (BERGER & BERGER, 1992). The observations are not homogeneous, because they were carried out in different years (1963-1989), on different populations and in various environments. The study areas are situated within the drainage of the Warta river in central Wielkopolska. This region forms a rather homogeneous geographical and climatic unit (BARTKOWSKI, 1970).

Newly metamorphosed froglets of both species, which are the most common amphibians in the region (BERGER, 1987), were gathered near water bodies in which they lived as tadpoles Individuals were killed and preserved in 3 % formaldehyde. They originated from the vicinity of Poznań and the Biological Field Station of our Institute in Turew (about 40 km south of Poznań), and from Jaskółki near Ostrów Wielkopolski (about 100 km south-east of Poznań). In two localities the froglets were gathered every two or three weeks, in the others they were caught during researches of water frogs. We were not able to gather any data on older brown frogs in nature, because we did not mark them and it was not possible to estimate their age by their body length.

During some years froglets were reared in captivity. The frogs were measured with an accuracy of 0.1 mm (snout-vent length) after capture and later on every spring until their death; some were measured twice in a year (fig. 2). We marked them collectively or individually by toe-clipping. In 1986, about 20,000 tadpoles of *Rana arvalis* were put into 10 basins which contained 200-350 l of water with plants and mud from a pond. The basins were located in a fenced enclosure of 10 × 10 m in a garden. These tadpoles and frogs never received any special food during their life-time. It was much easier to keep *Rana arvalis* than *Rana temporaria*. Froglets of the former species were more vital and usually survived some years in captivity, whereas those of the latter usually perished before or during the first hibernation.

RESULTS

GROWTH OF FROGS IN NATURE

After metamorphosis, the body length of *Rana arvalis* and *Rana temporaria* froglets was rather similar and averaged about 14-16 mm in the studied localities (fig. 1). In autumn, individuals of *Rana temporaria* were much larger than those of *Rana arvalis*: the first reached about 35 mm (range 24-42 mm), and the second about 24 mm (range 16-32 mm) in body length In other localities their body length in autumn was as follows: in Sława Wielkopolska near Poznań (Sept. 14, 1962), *Rana arvalis* measured 17.2-30.2 mm (mean 22.45, N – 53), and *Rana temporaria* 17.8-32.0 mm (mean 24.10, N = 19); in Zbęchy near Turew (Sept. 7, 1978), *Rana arvalis* measured 22.7-34.0 mm (mean 28.10, N = 17), and *Rana temporaria* 24 3-42.5 mm (mean 34.20, N = 28).

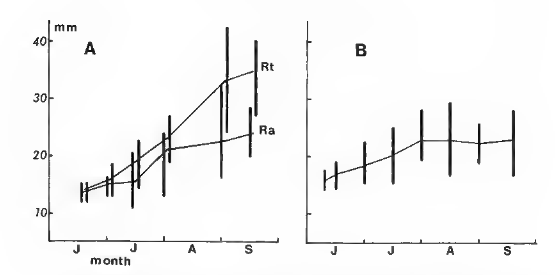


Fig. 1. — Growth of brown frogs in nature. (A) Rogaczewo near Turew, 1978: Rt, *Rana temporaria* (N = 59); Ra, *Rana arvalis* (N = 130); (B) Poznań-Naramowice, 1963. *Rana arvalis* (N = 512). Mean values of body length are joined by lines, extreme sizes of froglets are indicated by vertical bars.

Froglets after first hibernation were collected in two localities. In Rogaczewo (May 5, 1978), we gathered 66 froglets of *Rana arvalis* with body length of 18.7-31.0 mm (mean 25.12; see fig 1), and in Turew park (April 20, 1980), we caught froglets of both species. *Rana arvalis* measured 29.0-40.0 mm in body length (mean 35.70, N = 3), and *Rana temporaria* 36.0-52.0 mm (mean 44.40, N = 65).

GROWTH OF FROGS IN CAPTIVITY

Froglets from nature (fig. 2)

The froglets from Jaskółki which were caught in autumn (Sept. 5, 1988) were marked individually, and the others which were caught during metamorphosis were marked collectively. The following year, initially smaller individuals grew more rapidly than larger ones (Table I).

In summer after the first hibernation the sexual features appeared in all surviving frogs of both species, and after the second hibernation all frogs that were left released gametes. The three females of *Rana arvalis* from Poznań-Junikowo measured 41.5, 42.6 and 46.5 mm in body length and laid 141, 168 and 728 eggs respectively; those from Zbęchy measured 44.0, 49.5, 52.0 and 58.0 mm in body length and laid 692, 1077, 1434 and 2055 eggs respectively.

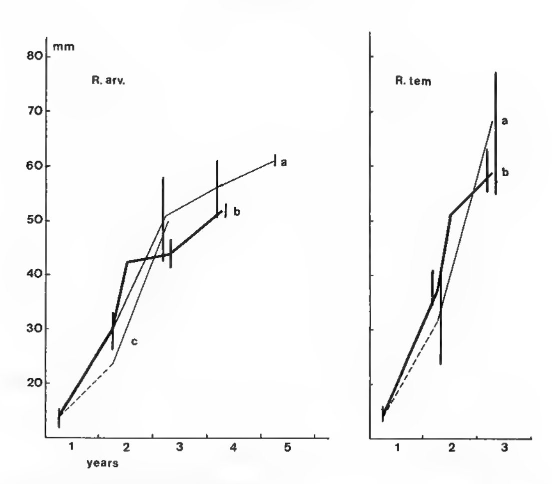


Fig. 2. — Growth of brown frogs in captivity.

R. arv., *Rana arvalis* from (a) Zbęchy near Turew, 1978 (N = 26); only two individuals survived the fifth hibernation; (b) Poznań-Junikowo, 1970 (N = 32), only two frogs survived the third hibernation; (c) Jaskółki near Ostrów Wielkopolski, 1988 (one female only).

R. tem., *Rana temporaria* from. (a) Jaskółki near Ostrów Wielkopolski, 1988 (N = 20); 11 frogs survived the first hibernation; (b) Poznań, 1974 (N = 30); only five frogs survived the second hibernation.

Individuals of *Rana arvalis* from Poznań-Junikowo and *Rana temporaria* from Poznań were measured twice in the second season Individuals from Jaskółki were removed from the rearing area on July 30.

Among *Rana temporaria* from Poznań only one female of 63.0 mm body length laid 972 eggs spontaneously with the only male which survived the winter. The other three females with body length of 56-58 mm were caught and after dissection we found mature ova in their ovisacs which were ready to be shed (627, 730 and 821 eggs).

The mean body length of *Rana arvalis* from Poznań-Junikowo reached 29.9 mm after the first hibernation, 42.3 mm in July, and 44.0 mm in the spring of the next year; this

Table I. — Growth of *Rana temporaria* individuals in captivity. The frogs are arranged according to body length at the day of capture in Jaskółki near Ostrów Wielkopolski.

Females					Males				
No	Body	length	Growth		No.	Body	length	Growth	
of frog	Sept. 5 1988	July 30 1989	mm	%	of frog	Sept. 5 1988	July 30 1989	mm	%
16	27.2	65.0	37.8	139	14	25.0	55.0	30.0	120
20	27.8	76.0	48.2	174	21	25.2	63.0	37.0	147
22	28.0	71.0	43.0	154	27	37.8	77.0	39.2	104
15	32.0	71.0	39.0	122	13	39.5	67.0	27.5	70
24	34.0	68.0	34.0	100	23	40.0	66.0	26.0	65
26	36 3	71.5	35.2	97					
Means	30.9	70.4	39.5	131		35.5	65.6	31.9	101

means that within the first period they grew about 12.4 mm and in the second only 1.7 mm. The *Rana temporaria* from Poznań grew in a similar way. They averaged 37.0 mm in body length after the first hibernation, 51.0 mm on June 12, and 58.5 mm in the spring of the next year. Within the first period they grew about 22.6 mm and in the second only 14.0 mm.

Froglets from tadpoles reared in captivity

On April 4, 1986, we brought 23 clumps of spawn of *Rana arvalis* from Poznań-Naramowice pond, which contained 1171-2563 eggs (mean 1737). Sixteen clumps were kept in containers outdoors, the others in the laboratory. During their development the temperature decreased to 5°C below zero at night. Percentage of embryos which reached tadpole stage was as follows: in the laboratory 78.6-97.2 % (mean 88.2 %), and outdoors 8.5-88.1 % (mean 38.1 %). All tadpoles were mixed and distributed into basins in the enclosure in Jaskółki in which there were no brown frogs before. The first metamorphosed individuals appeared on June 2, but in July tadpoles could still be seen in basins. At the end of September we caught three froglets (40-45 mm in body length) with typical male features (blue colour and nuptial pads).

The following year, the frogs formed a breeding aggregation in the largest basin, but by the end of April there were no eggs. In the basin we found 14 adult *Rana arvalis* males with 40.0-48.6 mm in body length (mean 44.8), but there were no females. Juvenile individuals were also found in the rearing area: 23 females with body length of 19.0-42.8 mm and 14 males with body length of 15.5-39.5 mm. The ovaries of these females were in bud stage, and in the testes of some juvenile males (33.0-39.5 mm in body length) moving spermatozoa were present.

DISCUSSION

The period of metamorphosis of tadpoles of *Rana arvalis* and *Rana temporaria*, which are explosive early spring breeders, is usually very short (VAN GELDER & OOMEN, 1970; RYSZKOWSKI & TRUSZKOWSKI, 1975). This suggests that individuals of these species should form compact age groups (KRIVOSHEYEV, OPENKO & SHABANOVA, 1960, GAJZAUSKIENE, 1966; HEUSSER, 1970). However, their growth rates in nature (VAN GELDER & OOMEN, 1970; LOMAN, 1978; CHMELEVSKAJA, 1985; fig. 1) and in captivity (SMIRINA, 1980, 1986) are quite variable and opinions on their age and sexual maturity are often contradictory.

The data of VAN GELDER & OOMEN (1970) and LOMAN (1978), and our results suggest that in nature only the yearlings can be identified by body length, whereas the division of older frogs into age classes is impossible by body length alone. KLEINENBERG & SMIRINA's (1969) skeletochronological studies corroborate this conclusion. They found that individuals of *Rana temporaria* caught near Moscow in June formed three size classes, but only one class which contained the smallest individuals (after the first hibernation) was homogeneous, whereas the other two classes contained individuals which were 2-9 years old. Other authors (MINA, 1974; ISHCHENKO & LEDENTZOV, 1985, 1986) obtained similar results.

Our observations show that individuals of both species grew most rapidly at the beginning of the second season (May-June, fig. 2, Table I), or before reaching maturity. Within yearlings of *Rana temporaria* in the Wielkopolska region, however, we observed considerable differentiation in autumn. Their mean body length ranged from 24.10 mm in Sława Wielkopolska to 44.40 mm in Turew (see SMIRINA, KLEVEZAL & BERGER, 1986). The conditions in our rearing area, in which the yearlings reached intermediate body lengths (fig. 1-2), are therefore likely to have been close to those in nature. To this extent, our observations on frogs in captivity can be compared with those obtained by others in wild.

Our data with regard to sexual maturity are also not always in agreement with other authors. Opinions on this problem are differentiated and data which are reported by many authors refer mostly to *Rana temporaria*.

According to GIBBONS & MCCARTHY (1984), in west Ireland most of the individuals in the breeding aggregations of *Rana temporaria* were two years old frogs (84 % of the males and 52 % of the females). In Britain also numerous such frogs were found in the breeding time (BEEBEE, 1980; COOKE, 1981). However, near Moscow in Russia (CHMELEVSKAJA, 1985), near Berne in Switzerland (RYSER, 1986, 1988) and in southern Sweden (LOMAN, 1976, 1978) so young individuals were extremely rarely present in breeding aggregations. Some others suggest that individuals of this species reach maturity in the third (HEUSSER, 1970; MINA, 1974) or in the fourth year (KLEINENBERG & SMIRINA, 1969). In our rearing (fig. 2, Table I), however, *Rana temporaria* reached maturity during the second season and after the second hibernation released gametes as two years old frogs. These results corroborate TOMASIK's (1969) opinion on Polish *Rana temporaria*.

The data on *Rana arvalis* are very scanty. ISHCHENKO & LEDENTZOV (1985, 1986) found that in the breeding populations near Sverdlovsk in Russia only few individuals

(about 1 %) were two years old. TOMASIK (1969) also supposes that in Poland *Rana arvalis* can reach maturity in the second year. According to our results all individuals of *Rana arvalis* in rearing reached maturity in the second season. Other authors suggested, however, that individuals of this species mature later: in southern Sweden in the third or in the fourth year (LOMAN, 1976, 1978), and in the Netherlands in the third year (VAN GELDER & OOMEN, 1970). The latter authors report, however, that at the end of the second season most frogs had more than 40 mm in body length and at that time it was possible to distinguish males from females This statement suggests that such frogs were mature and took part in the breeding aggregations as two years old animals.

The growth of *Rana arvalis* and *Rana temporaria* froglets in the first year is highly differentiated not only in Sweden (LOMAN, 1976, 1978), but also in the Netherlands (VAN GELDER & OOMEN, 1970), in Russia (CHMELEVSKAYA, 1985) and in Poland Near Poznań in every locality and nearly in every sample, including froglets after the first hibernation, there were very large and very small individuals. The situation was, however, reversed in the second year of their life: the growth of small froglets was much faster than that of large ones (Table I; see also VAN GELDER & OOMEN, 1970). Similar observations which are consistent with the "compensation growth phenomenon" in animals (MINA & KLEVEZAL, 1976) have been observed in some other anuran species (JAMESON, 1956, BERGER, 1970; PŁYTYCZ & BIGAJ, 1985; BERGER & RYBACKI, unpublished data), which suggests that this phenomenon may be rather common in Amphibia.

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Skin morphology in larval, paedomorphic and metamorphosed Alpine newts, *Triturus alpestris apuanus*

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Histological data on the skin of larval, paedomorphic and metamorphosed specimens of the Alpine newt, *Triturus alpestris apuanus*, have been analyzed and compared with natural history information. The skin of early larvae is composed of a few cell layers and contains Leydig cells, but lacks exo-epithelial dermal glands, which appear later during the premetamorphic stage. In metamorphosed newts the skin is typically multilayered and cornified, with abundant mucous and serous glands. The situation in paedomorphics, which are branchiate and live in water, is variable: in some cases (corresponding to immature "giant larvae"), the skin presents larval aspects (Leydig cells are present), while in others (usually sexually active and paedogenetic individuals) it is similar to that of metamorphosed newts. Thus metamorphosis in paedomorphic newts is not abandoned, but only delayed. The result is a mosaic of larval and metamorphosed characteristics, which can be related to the highly plastic ecology of these newts.

Introduction

The typical life cycle of urodeles is amphibious, with aquatic larvae and metamorphosed individuals living in a terrestrial habitat. Nevertheless, paedomorphism (sensu Dubois, 1987, or neoteny, sensu Breuil, in press, understood as the retardation in the development of somatic and/or gonadal features or as the achievement of the sexual maturity while retaining larval-juvenile characters) often occurs. Paedomorphic newts have external gills, reach a greater size than larvae, and are totally aquatic. The causes of paedomorphism are not well known, although ecological constraints are often invoked (Wilbur & Collins, 1973). Among the European urodeles, the Alpine newt, *Triturus alpestris* (Laurenti, 1768), throughout its range and involving most subspecies, shows a high incidence of paedomorphism. Recently, as a part of a wider study of paedomorphism in Italian Alpine newt populations (Andreone & Dore, 1991), some preliminary hypotheses on the ecological basis of the phenomenon were formulated, moreover,

information has been gathered on variations in the histology of thyroid glands and gonads of paedomorphic and metamorphosed individuals (ANDREONE, DORE & USAI, 1991). In the present paper, as a complement, data on skin morphology during the life cycle are presented, taking into account that the amphibian integument witnesses the changes from an aquatic to a terrestrial habitat, acting as a medium for ionic exchange (DUELLMAN & TRUEB, 1986; LODI et al., in press).

MATERIAL AND METHODS

The population studied (which belongs to the subspecies *Triturus alpestris apuanus*), inhabits an artificial temporary pond located between the towns of Murazzano and Bossolasco (southern Piedmont, north-western Italy), at an altitude of about 700 m, with a surface area of about 100 m^2 and a maximum depth of 1.5 m. No aquatic vegetation (except *Chara* algae) is present, while other amphibians living there are the common frog (*Rana temporaria*) and the common toad (*Bufo bufo*). The climate of the area is Mediterranean (MENNELLA, 1967), with maximum rainfall in May and November. In this site a remarkable number of aquatic newts are found in the water throughout the year, even during non-reproductive periods.

Each month, from January 1988 to December 1989, several aquatic newts were caught by hand-netting (ANDREONE & DORE, 1992). The following categories were examined:

(1) Larvae, i.e. branchiate individuals with a total length less than 40 mm, having a brownish-greenish back, scattered darker spots, and a whitish belly (fig. 1). Sometimes two age cohorts of larvae may co-exist in the same pond, resulting from two egg depositions at different times during a year (ANDREONE & DORE, 1992).

(2) Metamorphosed, i.e. sexually mature newts. Such individuals court during the breeding season, when they display secondary sexual characters (SSC), such as lateral white stripes, extended dorso-caudal crests and swollen cloacae in males, and a swollen abdomen and turgid cloaca in females (fig. 2). Although the metamorphosed aquatic population is mainly represented by adults, juvenile specimens also are found; such individuals are smaller than sexually mature individuals, do not show SSC and, in some cases, have a light dorsal line.

(3) Paedomorphics (sensu lato), i.e. individuals with external gills (more or less developed), larger body size than larvae and with a yellow-orange belly. We refer to "neoteny" or "paedomorphism" according to KOLLMANN (1884a-c) and DUBOIS (1987): "paedomorphic" to indicate branchiate newts without SSC and lacking the courtship behaviour, corresponding to the "partial neotenic" (sensu BREUIL, in press) (fig. 3), and "paedogenetic" (= "total neotenic") to indicate the branchiate newts displaying courtship behaviours and SSC (fig. 4).

After capture, newts were anaesthetized by immersion in a 0.5 % MS 222 Sandoz solution. Some individuals were dissected to verify their gonadal status, and a portion of their dorsal and ventral skin was cut off, fixed in cold buffered formalin, dehydrated, and infiltrated overnight with a glycol metacrylate monomer ("Technovit 7100" Kulzer) in a

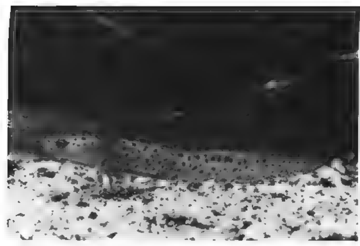


Fig. 1. — Larva of Alpine newt (*Triturus alpestris apuanus*), characterized by external gills, continuous caudal crests, and a light brownish scattered back.

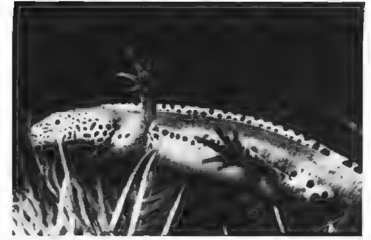


Fig. 2. — Metamorphosed male of Alpine newt during the breeding season, with well developed secondary sexual characters (SSC), e.g. dorsal and caudal crests, white lateral stripe, blue back coloration and swollen cloaca.

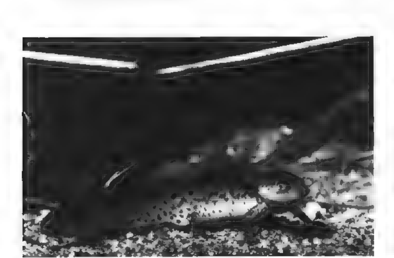


Fig. 3. — Paedomorphic (partial neotenic and sexually immature giant larva) Alpine newt, characterized by a general larval aspect, undeveloped gonads and by the absence of evident SSC.



Fig. 4. — Paedogenetic (totally neotenic) adult male of Alpine newt, with external gills and well developed secondary sexual characters.

ice bath. Polymerization was carried out at about 6-10°C to preserve enzymatic activities (Dore & Usai, 1986; Andreone & Dore, 1992). Skin sections of 2 µm were stained with acid fuchsin-toluidine blue (Dougherty, 1981), and with Burstone direct coupling method of substituted naphtols contrasted with methyl green for Alkaline Phosphatase (APH) activity (Mazzi, 1977; Loyda, Gossrau & Schleber, 1979). APH activity may be correlated with transcutaneous ionic transport (see Lodi et al., in press). Controls were carried out in the absence of substratum.

Results

Larvae

The larval skin is composed of a few cell layers (three or four) lying on a dense layer of dermis ("basement lamella", Fox, 1977). In all specimens examined the epithelial basal cells have an elongated nucleus, are irregular in shape, and penetrate with their apical part among the overhanging Leydig cells. The latter cells, well known in larvae and neotenic urodeles (see Hay, 1961; Raffaelli, 1989) have a poorly known function, but they probably are glandular (Fox, 1988) and secrete mucus into subsurface extracellular compartments of the epidermis (Duellman & Trueb, 1986). Leydig cells are large and rounded, with a clear cytoplasm, PAS-positive granules and prominent nucleus, and they occupy the entire epidermal thickness and press against the common epithelial cells with their convex surface. We did not observe a general uniformity in the morphology and disposition of cells in the skin of different larvae. However, in early larvae the Leydig cells are disposed in two layers (basal and sub-apical) and contain abundant PAS-positive granules, while in older larvae they form only one layer and have a scarce granulation. The skin surface is not cornified, and is composed of one or two layers of flattened epithelial cells, while isolated supporting cells are interposed among the underlying Leydig cells. Neuromasts of the lateral line are constantly present. In early larvae the dermis is thin and usually not invaded by glandular (serous and mucous) elements. In some large premetamorphic larvae or in larvae becoming paedomorphic (see later), precocious gland buds begin to differentiate (fig. 6). Low APH activity is seen, and — when visible — it is localized in the most external epithelial cells, whereas it is totally lacking in the dermis, except in the blood vessels of the subcutaneous layer.

Metamorphosed specimens

The epidermis of metamorphosed Alpine newts (either sexually mature or newly metamorphosed) is similar to that described for *Triturus carnifex* (Lodi, 1968; Lodi & Bani, 1971), being about four to six cells thick. Migration of deep cells occurs towards the surface. Cells of the basal or germinative layer are large and irregularly shaped, generally columnar or cuboidal, and arrayed in a low palisade. Mitoses are often visible in the germinative layer, and melanophores are commoner in the dorsal dermis than elsewhere, but some scattered melanophores may be found in the dorsal epidermis (Duellman & Trueb, 1986). Flask cells (involved in the sodium transport; see Fox, 1986a-b; Zaccone



Fig. 5. — Histological structure of the skin in an Alpine newt larva. Two layers of Leydig cells with PAS-positive granules are evident, together with plate superficial cells. Toluidine blue coloration. The bar corresponds to 10 µm.

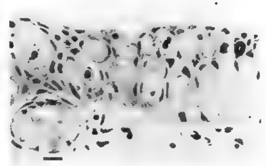


Fig. 6. — Late larval skin, with Leydig cells, with a dermal gland bud. Toluidine blue coloration. The bar corresponds to 10 µm.



Fig. 7. — Histological structure of the skin in a metamorphosed Alpine newt. Great exo-epithelial glands are visible, together with several cell layers and a cornified surface. Toluidine blue coloration. The bar corresponds to 10 µm.

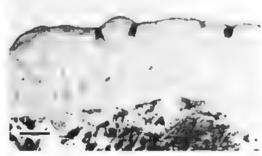


Fig. 8. — Metamorphosed skin, with flask cells shown by means of APH and Burstone's method of substituted naphtols contrasted with methyl green. The bar corresponds to 10 µm.

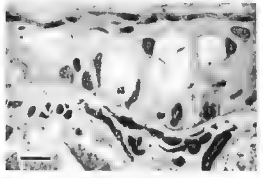


Fig. 9. — Structure of the skin in a paedomorphic immature Alpine newt. Empty Leydig cells (disposed in a single layer) with scarce PAS-positive granules are visible. Mitoses are evident in the cells among Leydig cells. Toluidine blue coloration. The bar corresponds to 10 µm.



Fig. 10. — Skin of a paedogenetic Alpine newt the structure and morphology — even with a reduced superficial cornification — is similar to the metamorphosed one. Toluidine blue coloration. The bar corresponds to 10 µm.

et al., 1986) are distributed more or less regularly in the subcorneous layer (fig. 7). The skin surface is cornified, with protruded warts, two or three flattened cells thick. Highly developed mucous and serous glands are always evident in the spongy thick dermis just below the epithelium either in the ventral or in the dorsal skin. In metamorphosed newts, APH activity is similar in the dorsal and in the ventral skin, varying from low to moderate in the cells of corneous and subcorneous layers, but lacking in the deepest layers. Flask cells usually show an intense APH activity (fig. 8) in their neck region. In the dermis, a high APH activity is evident, particularly in the blood vessels and around and inside the glands.

Paedomorphic newts

In paedomorphic newts, skin morphology is rather variable and not easily generalized. In animals showing non-functional gonads and without evident SSC, histological organization is roughly intermediate between that of larval and metamorphosed skin. The superficial cells are relatively loose (somewhere separated by empty spaces), neuromasts can be seen (even if rarer than in larvae), large exo-epithelial glands are present, and Leydig cells (Fox, 1988) are not so orderly as in larvae, have a lighter cytoplasm, scarce granules. In these animals mitoses can be observed in cells interposed among Leydig cells (fig. 9).

In some paedomorphic specimens (Gabrion & Sentein, 1986), a mosaic situation can be noticed: the Leydig cells are still present and abundant in some areas, while in others they are absent. In other branchiate newts (sexually mature and paedogenetic), the skin is highly similar to that of the metamorphosed ones, although with a lower surface cornification (fig. 10). It is sometimes possible to observe empty spaces inside the epithelium, probably derived from the degeneration of Leydig cells. APH activity in the more superficial layers is usually scarce or even absent, whereas in the dermis it is localized in the portion just below the basal layer of the epithelium and more evident around the glands than elsewhere. Flask cells are present in a few paedogenetic specimens and show a high APH activity in the portion superficial to the nucleus.

Discussion

Analysis of the organization of skin of Alpine newts throughout the life cycle discloses high variability from larvae to metamorphosed specimens. The skin of paedomorphic specimens has a structure which varies from larval-like to metamorphosed-like organization, sometimes the skin shows both larval characteristics (e.g. Leydig cells, neuromasts) and metamorphosed characteristics (e.g. dermic glands, horny surface). This intermediate situation is also evident in the activity of APH, as has been stressed by Lodi et al. (in press). These data mainly agree with Gabrion & Sentein (1976), who observed that Leydig cells vary in number and shape from totally larval to late paedomorphic palmate newts (*Triturus helveticus*), in which the skin closely resembles that of meta-

morphosed individuals. In late paedogenetic Alpine newts, the skin rearrangement extends also to the empty spaces left by the disappearance of the Leydig cells. The mitoses observed in the intermediate epithelium can be interpreted as part of a regenerative process.

The variability reported suggests the persistence (and not the arrest) of metamorphic phenomena even in paedomorphic Alpine newts, and stresses the difficulty in categorizing branchiate newts. In fact, by external examination it is possible to observe sexually inactive paedomorphic individuals which resemble larvae, and others (paedogenetic) which exhibit a typical mating coloration (more visible in males), and are sexually active, behaving during the courtship like the typical metamorphosed adults (ANDREONE, 1990; BOVERO, 1991). Natural history observations confirm that gill development and length vary among paedomorphic individuals of the very same population and that these may — at a certain moment of their life — metamorphose. Thus, it is not possible to consider the paedomorphics as a whole and uniform category, because they are part of a continuum, from larval to metamorphosed newts. In this sense it is worth reaffirming, in contrast to MOLA & BERTOLANI (1981), that the presence of Leydig cells is not a constant neotenic character. Perhaps these authors analyzed the skin of larval-like paedomorphic newts ("giant overwintered larvae") and not that of the typical paedogenetic newts. The correlation between morphology and function of the skin has been pointed out by LODI et al. (in press): a typical larval organization (with Leydig cells) is characterized by the absence of active sodium transport, that is evident when the epidermis acquires, at least in part, the metamorphosed characteristics.

All these considerations indicate how the newt life cycle (and more particularly that of *Triturus alpestris*) cannot be rigidly schematized, but that plasticity in life history characteristics is itself adaptive. In fact, the highly aquatic Alpine newts synchronize their permanence in the water both to altitude differences (and thus to the duration of their breeding period) and to desiccation-rain alternation in unpredictable habitats (ANDREONE et al., in press). An outline of the life history of Alpine newts is represented in fig 11 — to interpret the observations on skin morphology and on external features (modified from VERRELL, 1985, DŽUKIĆ et al, 1991, and ANDREONE et al., in press). Typically, newts spend the first part of their existence as aquatic larvae; thereafter metamorphosis induces some drastic changes (e g. in the skin structure) as the newts become terrestrial. Growth until sexual maturity occurs on land (particularly when the breeding site is temporary), but juveniles may return to water for non-reproductive reasons, as has been reported for other species by VERRELL (1985) and ANDREONE & GIACOMA (1989). Nevertheless an alternative strategy is observed in more stable habitats: adulthood is reached through a direct aquatic development, and larvae may overwinter in the water (especially those hatched in autumn, see ANDREONE & DORE, 1992), becoming paedomorphic juveniles Later they may become paedogenetic, thus displaying SSC and courtship patterns. Throughout this aquatic development, skin morphology modifies, although the changes are not so drastic as in the transformation from water to land in the typical life cycle. For this reason a mosaic situation is seen, and neotenic individuals may present both larval and metamorphosed characters. Nevertheless metamorphosis may occur any time in the aquatic development Thus, metamorphosed individuals found in the water may result either after growth in a terrestrial habitat, or by transforming at various stages of their aquatic life, possibly on

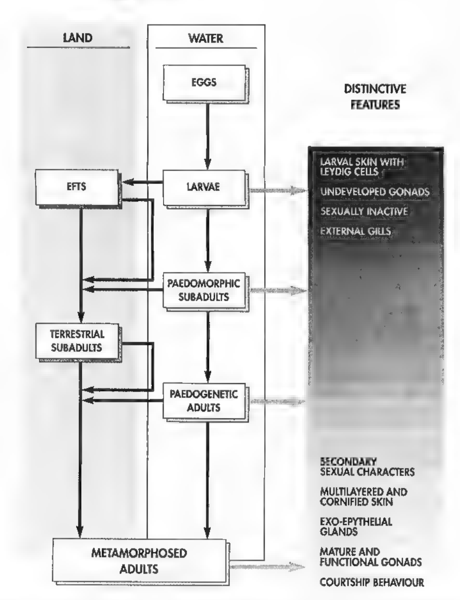


Fig. 11. Outline of the life cycle in *Triturus alpestris apuanus*. The left part summarizes the passages between water and land throughout growth and metamorphosis. The black arrows indicate habitat changes, but do not suggest any preference, depending on the characteristics and constraints of each individual and population The right part summarizes some typical features of the extreme categories of aquatic newts, from an early larval stage to a final paedogenetic-metamorphosed phase. Grey arrows indicate the correspondence of phases and biological features. Between these two extremes several intergrades are present. The distinctive features of metamorphosed newts, excluding the characteristics related with sexual activity, are common both to adults and to juveniles.

the occasion of pond desiccation or of water pollution (see Andreone & Dore, 1991). The reasons for this plasticity should be sought in adaptive explanations: paedomorphism – as remarked by Breuil (in press) – may lead to earlier sexual maturity, life in a more stable aquatic habitat, or better utilization of trophic resources (Kalezić, Džukić & Tvrtković, 1990).

Résumé

L'histologie de la peau du triton alpestre (*Triturus alpestris apuanus*) a été analysée et comparée avec des données concernant l'histoire naturelle de l'espèce. La peau des jeunes larves est composée d'un nombre réduit de couches cellulaires et contient des cellules de Leydig, mais pas de glandes extra-épithéliales; celles-ci deviennent visibles plus tard, avant la métamorphose. Chez les tritons métamorphosés elle est typiquement pluri-stratifiée et cornifiée, avec beaucoup de glandes muqueuses et séreuses. L'organisation épithéliale des individus pédomorphiques, qui ont des branchies et vivent dans l'eau, est assez variable: chez certains exemplaires (correspondant aux larves géantes immatures), la peau présente des caractéristiques typiquement larvaires (par exemple les cellules de Leydig sont encore présentes), tandis que chez d'autres (habituellement sexuellement actifs bien que pédogénétiques) elle est très similaire à celle des tritons métamorphosés. Ainsi, chez les tritons pédomorphiques, la métamorphose n'est pas abolie, mais seulement retardée. Il en résulte une mosaïque de caractéristiques larvaires et post-larvaires. Ces caractéristiques sont en relation avec l'écologie plastique du triton alpestre.

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Contents

Jordi Serra-Cobo
Descripción de una nueva especie europea de rana parda (Amphibia, Anura, Ranidae) 1

Leszek Berger & Mariusz Rybacki
Growth and maturity of brown frogs, *Rana arvalis* and *Rana temporaria*, in central Poland 17

Franco Andreone, Bruno Dore, Pasquale Usai & Adriana Paraninfo
Skin morphology in larval, paedomorphic and metamorphosed Alpine newts, *Triturus alpestris apuanus* 25

Miscellanea

Application for membership of ISSCA and/or subscription to *Alytes* 16

Alain Dubois
Dates de publication du journal *Alytes* (1992) 26

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